**PATENT APPLICATION**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of

Terry J. AMISS, et al.

Docket No: A9272

Application No.: 10/721,091

Group Art Unit: 1641

Confirmation No.: 6187

Examiner: David J. Venci

Filed: November 26, 2003

For: COMPOSITIONS AND METHODS FOR MEASURING ANALYTE CONCENTRATIONS

**DECLARATION UNDER 37 C.F.R. § 1.132**

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, J. BRUCE PITNER, hereby declare and state:

1) THAT I am a citizen of the United States and reside in Durham, North Carolina;
2) THAT I have received the degree of Ph.D. in organic chemistry from The University of Vermont;
3) THAT I have been employed by Becton Dickinson and Company since 2000, where I hold a position as Technology Manager, with responsibility for developing biosensors based on engineered proteins, novel fluorophores and biocompatible polymers;
4) THAT I have reviewed the Office Action dated December 8, 2006.
5) THAT the following data was generated by my laboratory under my direction and supervision. The data presented addresses the construction of fusion proteins for purposes of analyte detection using the methods of the above-captioned invention wherein the fusion protein comprises a functional periplasmic binding protein (PBP), fused to at least one fluorescent protein, and at least one labeling moiety.

6) In the experiment used to generate figures 1 and 2 of this Declaration, the PBP is the L238C mutant of GGBP, and the fluorescent protein is the discosoma red fluorescent protein (DsRed2). The labeling moiety is the cysteine-reactive fluorescent dye acrylodan. A C119A mutation was introduced in the DsRed2 sequence ("C119A" indicates a cysteine to alanine mutation at amino acid position 119 in the DsRed2 wild-type amino acid sequence). The sensor was exposed to normal saline, or normal saline containing glucose. The emission spectra of the mutated DsRed2 was compared to the emission spectra of unmutated DsRed2 (i.e., cysteine-containing dsRed2 or wild-type dsRed2) as discussed below.
7) In Figure 1, below, the spectra shows dsRed2 emission as a result of analyte binding to the PBP portion of the wild-type dsRed2 - GGBP fusion protein. A comparison of the two curves reveals that unmutated dsRed2 emission (nm) changes little, if at all, when 10μM glucose is present, as compared to emission in the absence of analyte. It is surprising that the resulting fusion protein has no apparent change in FRET ratio in its fluorescence emission when the analyte glucose is added (Figure 1, No Glucose v. 10μM Glucose).
8) In contrast, when we introduced the C119A mutation into the dsRed2 fluorescent protein sequence, a change in FRET of the acrylodan-labeled fusion protein was measured that corresponded to addition of the analyte glucose (Figure 2., compare saturated versus no glucose). This result indicates that the C119A mutation of dsRed2 is useful to obtain dynamic ranges in the ratiometric methods that can be used to detect the presence of analyte.

**FIGURE 1**

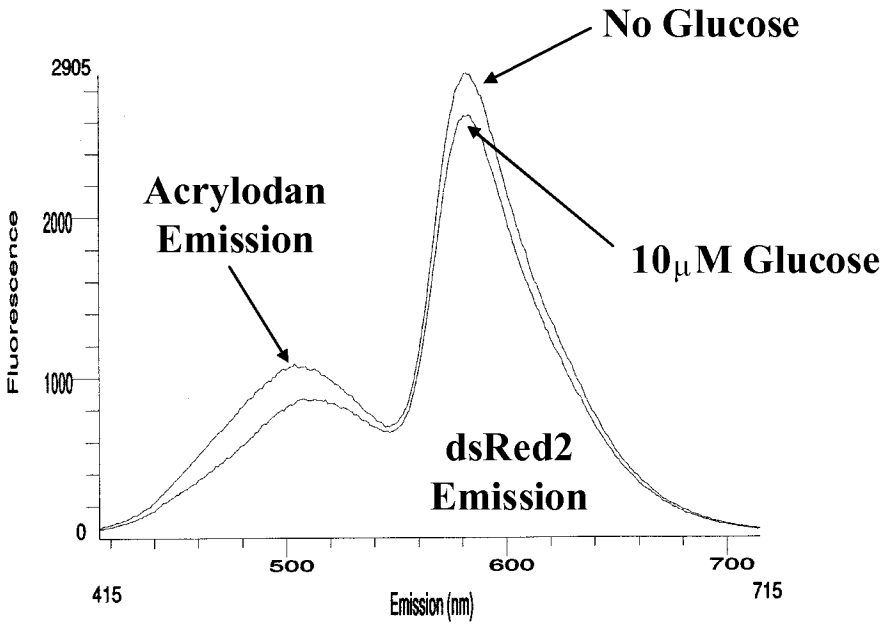
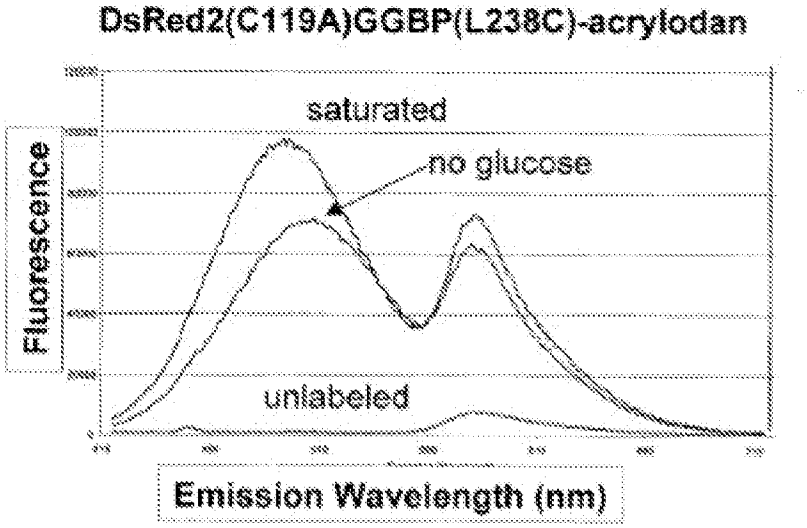

No Glucose
10μM Glucose
Acrylodan Emission
dsRed2 Emission
Fluorescence
2905
2000
1000
0
415
500
600
700
715
Emission (nm)

**FIGURE 2**




9) In my opinion, it would not have been obvious to one of skill in the art that mutation of C119, or any other cysteine, would provide meaningful and useful changes in FRET of the acrylodan-labeled fusion protein since our discovery of this phenomenon required exhaustive experimentation. After exhaustive study of the various residues, we now understand that this unexpected result was due to acrylodan binding at the C119 position of dsRed2, thus obscuring the FRET response to glucose obtained by acrylodan binding at L238C of GGBP. In other words, the presence of cysteine residues in dsRed2 can lead to overlabeling with dyes, such as acrylodan.

10) Nothing in the cited references teaches or discloses that mutation of C119, or any other cysteine, would provide dynamic ranges in the dye emission ratios that are necessary for useful biosensor output.

11) I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: MAY 8, 2007

J. Bruce Pitner